Docket No. 2251/76935/JPW/GJG/REB

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Yingru Wu, et al. Examiner: Stuart F. Baum

U.S. Serial No.: 10/594,785 Group Art: 1638

Filed : September 17, 2007

For : GENES INVOLVED IN PLANT FIBRE DEVELOPMENT

30 Rockefeller Plaza
New York, New York 10112

BY EFS
Commissioner for Patents
Alexandria, VA 22313-1450

SIR:

DECLARATION UNDER 37 C.F.R. §1.132 OF DANNY J. LLEWELLYN

I, Danny J. Llewellyn, hereby declare that:

1. I am named as an inventor on the above-identified patent application.

2. I am presently a Sub-Program Leader of Genomics and Plant Development at the Commonwealth Scientific and Industrial Research Organisation, Black Mountain Laboratories, Clunies Ross St, Black Mountain ACT 2601, Australia. A copy of my curriculum vitae is attached hereto as **Exhibit A**.

3. I have reviewed and am familiar with the subject application, including pending claims 117-142, which are attached here to as **Exhibit B**.

4. The subject application discloses, e.g. in Examples 5, 6, and 14, that *GhMYB25* and *GhMYB25-like* encode MYB proteins that are 69% identical to each other on the amino acid level, both have similar gene expression profiles in wild-type plants, and both have reduced expression in a cotton mutant with reduced fibre production.

5. In Example 12 of the subject application, the function of *GhMYB25* is analyzed and also represents the expected function of *GhMYB25-like*.

6. Example 12 of the subject application describes experiments that show transgenic tobacco plants over-expressing a *GhMYB25* transgene have increased trichome production on their leaves.

7. Example 12 of the subject application also shows the amount of increased trichome production on the leaves of transgenic tobacco plants correlates with the amount of over-expression of a *GhMYB25* transgene in the transgenic tobacco plants.

8. Increased trichome production on the leaves of transgenic tobacco plants over-expressing a *GhMYB25* transgene indicates that such transgene would cause increased fibre initiation and/or elongation if over-expressed in a fibre producing plant.

9. Transgenic tobacco plants over-expressing a *GhMYB25* transgene having increased trichome production on their

leaves is predictive that a *GhMYB25-like* transgene would cause increased fibre initiation and/or elongation if over-expressed in fibre producing plants.

10. Example 13 of the subject application describes how a transgenic fibre producing plant over-expressing a *GhMYB25-like* transgene may be created.

11. We have confirmed through experiments that the predictions within the subject application, in particular Example 13, were correct. The data discussed below was obtained by me or individuals working under my guidance and supervision.

12. The experiments discussed below in paragraphs 13-20, were performed to determine whether over-expressing *GhMYB25-like* in a transgenic fibre producing plant results in a transgenic plant having increased fibre initiation and/or elongation compared to wild-type plants.

13. The coding region of the D-genome homologue of *GhMYB25-like* driven by either a seed-specific (ovule epidermis) promoter, *FBP7* from petunia, or a constitutive viral promoter, *Stunt7*, were successfully introduced into cotton cultivar Coker 315 by *A. tumefaciens*-mediated transformation. The D-genome homologue of *GhMYB25-like* encodes a polypeptide also encoded by a nucleotide sequence which is identical to the nucleotide sequence set forth in SEQ ID NO: 38 and described in the subject application.

14. A total of 11 and 36 independent T_0 lines were produced for the *FBP7-GhMYB25-like* and the *S7-GhMYB25-like* constructs, respectively and T_1 seed harvested from 10 independent *FBP7-GhMYB25-like* and 2 independent *S7-GhMYB25-like* transgenic lines.

15. Vegetative growth of the T_1 plants appeared to be the same as wild-type plants.

16. Initial scanning electron microscope (SEM) analysis was performed on T_2 ovules (ovules produced on T_1 plants) from those transgenic lines as available. The number of fibre initiations was counted from SEM images in the middle of 0 dpa ovules and approximately 12 ovules were imaged per line, from multiple plants from each line.

17. The ovules from *FBP7-GhMYB25-like* plants showed an average increase in the number of fibre initiations between 13% and 28% in five independent lines compared to wild-type controls, with some individual ovules having 30-50% more fibre initiations.

18. Of the two *S7-GhMYB25-like* transgenic lines analyzed, one showed an average increase of 13% and the second showed a 22% increase in the number of fibre initiations compared to wild-type controls, again with a number of ovules having 60% more fibre initiations.

19. These experiments demonstrated that the increased production by a fibre producing plant of a polypeptide comprising consecutive amino acids whose sequence is identical to the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 38 of the subject application results in increased fibre initiation and/or elongation in the fibre producing plant.

20. These experiments also demonstrate the creation of a transgenic fibre producing plant with increased fibre initiation and/or elongation by genetically manipulating the plant such that the production of a polypeptide comprising consecutive amino acids whose sequence is identical to the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 38 of the subject application is increased when compared to a wild-type fibre producing plant.

21. Example 5 and Table 4 of the subject specification show that expression of the *GhMYB25* and *GhMYB25-like* genes is reduced in a mutant fibre producing plant with reduced fibre initiation and/or elongation in the plant.

22. A mutant fibre producing plant with reduced expression of the *GhMYB25-like* gene having reduced fibre initiation and/or elongation is predictive that reduction of the gene in a wild-type plant would cause reduced fibre initiation and/or elongation in that plant.

23. Example 13 of the subject application describes how a transgenic fibre producing plant with reduced expression of the *GhMYB25-like* gene may be created.

24. We have confirmed that reduced expression of *GhMYB25-like* results in reduced fibre initiation and/or elongation in fibre producing plants. The data discussed below was obtained by me or individuals working under my guidance and supervision.

25. The experiments discussed below in paragraphs 26-32, were performed to determine whether reducing the expression of *GhMYB25-like* in a transgenic fibre producing plant results in a transgenic plant having reduced fibre initiation and/or elongation compared to wild-type plants.

26. A constitutive 35S promoter-driven RNAi construct targeted against both the A and D-genome homologues of *GhMYB25-like* was introduced into cotton cultivar Coker 315 by *A. tumefaciens*-mediated transformation, and seven independent transgenic lines were produced.

27. Detailed phenotypic analysis was performed on four independent *GhMYB25-like* silenced (25Li) lines.

28. For each line, T_3 plants homozygous for the presence of the transgene or wild-type segregants of the same generation were analyzed.

29. The transcript levels for *GhMYB25-like* in these plants were significantly reduced in the ovules and seeds of all the silenced plants compared to their corresponding wild-type controls.

30. The seeds from the T_3 homozygous 25Li plants were essentially fibreless, except for a few fibres at the chalazal end, when compared to the normal fibred seeds from the wild-type plants. Fuzz fibres were also completely absent from all 25Li plants. The *GhMYB25-like* silenced plants were indistinguishable from their wild-type controls in respect to observable morphological features such as leaf shape, height, flowering time and flower structure.

31. These experiments demonstrated that the reduced production by a fibre producing plant of a polypeptide comprising consecutive amino acids whose sequence is identical to the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 38 of the subject application results in reduced fibre initiation and/or elongation in the fibre producing plant.

32. These experiments also demonstrate the creation of a transgenic fibre producing plant with reduced fibre initiation and/or elongation by genetically manipulating the plant such that the production of a polypeptide comprising consecutive amino acids whose sequence is identical to the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 38 of the

subject application is reduced when compared to a wild-type fibre producing plant.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the subject application or any patent issuing thereon.

Dated: 3/1/11

Danny J. Llewellyn

Curriculum Vitae

Danny James Llewellyn, PhD, FTSE

Professional Address: CSIRO Plant Industry
P.O. Box 1600
Canberra , ACT 2601

Nationality: Australian

Date and place of birth: 21/7/1954 Newcastle, NSW, Australia

Current Position: Group and Project Leader in Cotton Biotechnology, CSIRO Plant Industry, Canberra

Academic record/awards:

1975	BSc (Hons) Australian National University. Majoring in Biochemistry
1980	PhD Australian National University. Thesis on multifunctional enzymes
1994	Australian Cotton Grower's Research Association Researcher of the Year
1995	CSRO Plant Industry Chief's Award (Excellence in Science Delivery)
2000	Elected a Fellow of the Australian Academy of Technological Sciences and Engineering (ATSE) for contributions to Australian Agricultural Biotechnology
2001	Centenary Medal for services to Agricultural Biotechnology
2003	CSIRO Plant Industry Chief's Award (Excellence in Research Adoption)
2003	CSIRO Chairman's Medal
2006	ATSE Clunies Ross Award
2009	Weed Science Society of America Award of Excellence

Employment record:

1995 to present:	CSIRO Plant Industry (cotton biotechnology group and project leader)
1993 to 1999	Sub-program Leader Cotton CRC
1985 to 1995	Research Scientist, CSIRO Plant Industry Canberra
1982 to 1985:	Rural Credits Research Fellow, CSIRO Plant Industry, Canberra
1980 to 1982:	CSIRO Postdoctoral Fellow, Max-Plank Institute for Plant Breeding, Cologne, Germany
1980 to 1980:	Research Assistant, Dept Biochemistry, ANU, Canberra – while completing PhD thesis

Professional societies

Australian Society for Biochemistry and Molecular Biology
Australian Academy of Technological Sciences and Engineering

Refereed Publications:

Llewellyn, D.J., and Smith, G.D. (1978) An evaluation of active enzyme centrifugation as a zonal and boundary technique by the analysis of simulated data. *Arch Biochem Biophys.* **190**:483-94.

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Exhibit A

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Wu, Y., Rozenfeld, S., Defferrard, A., Ruggiero, K., Udall, J.A., Kim, H-R., Llewellyn, D.J and Dennis, E.S. (2005) Cycloheximide treatment of cotton ovules alters the abundance of specific classes of mRNAs and provides a method of generating novel ESTs for microarray expression profiling. *Molecular Genetics and Genomics* **274**: 477-493.

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Granted Patents and Patent Applications:

WO2005095614-A1; AU2005229157-A1; US2008196120-A1 Altering fiber initiation and/or elongation in a fiber producing plant comprises manipulating the plant by modifying a transcription factor, regulatory protein, or a cell cycle protein, produced at or around anthesis.

WO200245485-A; WO200245485-A1; US2002116736-A1; AU200220363-A; EP1349446-A1; BR200115782-A; CN1479569-A; ZA200303930-A; AU2002220363-B2; CN1326996-C; US7498492-B2; IN200300565-P2 Altering fibre development or properties of a fibre producing plant by modulating sucrose synthase activity and/or expression in such plants, useful for enhancing fibre yield and quality and for increasing seed size.

US5837849-A Isolated DNA that activates or enhances transcription from plant genes - effective in mono- or di-cotyledonous plants, particularly for increasing expression of heterologous genes.

US5710267-A DNA construct containing plant enhancer bound by octopine synthase transcription factor - and a heterologous plant promoter and gene, useful for generating transgenic plants for increased production of heterologous proteins.

AU9539013-A; AU715462-B Eucalyptus reproductive genes - useful for prodn. of sterile Eucalyptus trees useful for establishing wood lot plantations or in re-forestation projects.

WO9521924-A; EP746616-A; WO9521924-A1; AU9516611-A; EP746616-A1; EP746616-A4; AU692228-B; US6054318-A New glucose oxidase gene constructs - used for transforming organisms, esp. plants, to increase resistance to pests or diseases.

EP278659-A2; EP278659-A; ZA8800319-A; JP63276492-A; CA1309365-C; EP278659-B1; DE3853840-G; ES2074435-T3 Transcription activating element for plant genes - obtd. from octopine synthase (OCS) gene of T-DNA from Agrobacterium tumefaciens.

Listing of Claims:

1-116. (Cancelled)

117. A method of increasing fibre initiation and/or elongation in a fibre producing plant comprising genetically manipulating the plant such that the production of a polypeptide comprising consecutive amino acids whose sequence is identical to the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 38 is increased when compared to a wild-type fiber producing plant.

118. A method of reducing fibre initiation and/or elongation in a fibre producing plant comprising genetically manipulating the plant such that the production of a polypeptide comprising consecutive amino acids whose sequence is identical to the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 38 is reduced when compared to a wild-type fiber producing plant.

119. The method of claim 117 or 118, wherein the polypeptide comprises consecutive amino acids whose sequence is set forth in SEQ ID NO: 12.

120. The method of claim 117, wherein the genetic manipulation comprises exposing the plant to a vector which comprises a nucleotide sequence encoding a polypeptide comprising consecutive amino acids whose sequence is identical to the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 38.

121. The method of claim 118, wherein the genetic manipulation comprises exposing the plant to an antisense

polynucleotide or a catalytic polynucleotide which hybridizes to an mRNA molecule encoding the polypeptide, and/or exposing the plant to a dsRNA molecule that specifically down-regulates levels of an mRNA molecule encoding the polypeptide, such that the level of the polypeptide produced by the plant is reduced.

122. The method of claim 121, wherein the genetic manipulation is exposing the plant to the dsRNA molecule and the dsRNA molecule is double-stranded over at least 19 basepairs whose sequence corresponds to a consecutive sequence set forth in SEQ ID NO: 38, or to a consecutive sequence which is identical to the sequence set forth in SEQ ID NO: 38.

123. The method of claim 117 or 118, wherein the plant is a species of the Genus *Gossypium*.

124. A process of assessing the potential of a fibre producing plant to produce fibre, the process comprising analyzing whether the plant has a polynucleotide comprising a sequence identical to the nucleotide sequence set forth in SEQ ID NO: 38, and/or analyzing whether the plant is capable of expressing a polypeptide comprising an amino acid sequence which is identical to the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 38.

125. The method of claim 124, wherein the polypeptide comprises consecutive amino acids whose sequence is set forth in SEQ ID NO: 12.

126. A substantially purified and/or recombinant polypeptide selected from the group consisting of a polypeptide

comprising consecutive amino acids whose sequence is set forth in SEQ ID NO: 12.

127. An isolated and/or exogenous polynucleotide comprising a polynucleotide selected from the group consisting of:

i) a polynucleotide comprising consecutive nucleotides whose nucleotide sequence is set forth in SEQ ID NO: 38; and

ii) a polynucleotide which encodes a polypeptide comprising consecutive amino acids whose sequence is identical to the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 38.

128. A polynucleotide which is:

i) a catalytic polynucleotide capable of cleaving a polynucleotide whose sequence is identical to the nucleotide sequence set forth in SEQ ID NO: 38, or

ii) a dsRNA molecule comprising a polynucleotide which is double-stranded over at least 19 basepairs whose sequence corresponds to a consecutive sequence set forth in SEQ ID NO: 38, or to a consecutive sequence which is identical to the sequence set forth in SEQ ID NO: 38.

129. A vector comprising or encoding the polynucleotide of claim 127.

130. A vector comprising or encoding the polynucleotide of claim 128.

131. A plant or bacterial cell comprising the vector of claim 129.

132. A host cell comprising the vector of claim 130.

133. A transgenic plant, the plant having been transformed with the polynucleotide of claim 127.

134. A transgenic plant, the plant having been transformed with the polynucleotide of claim 128.

135. The transgenic plant of claim 133, which when compared to an isogenic non-transgenic plant, produces a modified level of a polypeptide comprising consecutive amino acids whose sequence is identical to the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 38.

136. The transgenic plant of claim 135, wherein the polypeptide comprises consecutive amino acids whose amino acid sequence is set forth in SEQ ID NO:12.

137. A transgenic seed of the plant of claim 133.

138. A transgenic seed of the plant of claim 134.

139. A process for producing fibre comprising obtaining the transgenic plant of claim 133 so as to thereby produce the fibre.

140. A process of breeding a fibre producing plant having a

polypeptide comprising consecutive amino acids whose sequence is identical to the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 38, the process comprising analyzing the plant for a genetic variation in a polynucleotide whose sequence is identical to the nucleotide sequence set forth in SEQ ID NO: 38, and/or analyzing the plant for a genetic variation in a polypeptide which is identical to the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 38, and breeding the fibre producing plant.

141. A process of selecting from a breeding population a fibre producing plant with altered fibre initiation and/or elongation potential, the method comprising:

i) crossing two plants which have differing potential to produce fibre so as to produce progeny plants,

ii) performing on the progeny plants a process comprising analyzing the plant for a genetic variation in a polynucleotide whose sequence is identical to the nucleotide sequence set forth in SEQ ID NO: 38, and/or analyzing the plant for a genetic variation in a polypeptide whose amino acid sequence is identical to the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 38, and

iii) selecting a progeny plant with altered fibre initiation and/or elongation potential when compared to a parent plant.

142. A process for identifying an agent which reduces fibre

initiation and/or elongation of a fibre producing plant, the method comprising:

i) exposing a polynucleotide comprising consecutive nucleotides whose sequence is identical to the nucleotide sequence set forth in SEQ ID NO: 38 to a candidate agent, and

ii) assessing the ability of the candidate agent to hybridize and/or cleave the polynucleotide.